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# Hepatic elastin content is predictive of adverse outcome in advanced fibrotic liver disease

Short running title – Hepatic elastin content predicts adverse outcome

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## Conflict of Interest

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## Abstract

**Aims.** Needle biopsy remains essential for diagnosis in assessment of liver disease, although there remains associated risk. Examination is largely limited to subjective evaluation and biopsies are not exploited to provide personalised prognostic information. Elastin is a durable component of fibrotic matrix in chronic disease, conferring resistance to remodelling and potentially influencing tissue biomechanics linked to portal hypertension. We hypothesised that elastin content was predictive of clinical outcome and so could be quantified to increase the beneficial information yield from a liver biopsy. **Methods and results.** Elastin content in liver biopsies was determined by image analysis, technically validated in an independent centre, and correlated with outcome in patients with advanced (Ishak stage  $\geq 5$ ) chronic hepatitis C virus-related chronic liver disease. Elastin was robustly quantified in an operator- and laboratory-independent manner, with very strong correlation of elastin staining measured by two methods of image classification ( $r_s = 0.873$ ,  $p < 0.00001$ ). Elastin content (but not absolute scar content or Ishak stage) was predictive for future clinical outcomes. In a cohort of patients without sustained virologic response, median hepatic elastin content was 3.4%, and 17 patients (57%) progressed to a liver-related clinical outcome; 11 of the 15 patients (73%) with hepatic elastin  $>3.4\%$  progressed to a clinical outcome, compared to only 6 out of 15 (40%) with elastin  $<3.4\%$ . The difference in time to outcome was significant. **Conclusions.** We describe a simple and reproducible method for elastin quantification in liver biopsies that provides potentially valuable prognostic information to inform clinical management.

**Keywords:** cirrhosis; prognosis; hepatitis C virus; elastin

Whilst the safety of needle biopsy of the liver is increasing,[1] there remain non-zero mortality and morbidity rates even with image-guided procedures,[2] and biopsy remains essential for diagnosis and assessment of liver disease.[3] However, in contrast to the use of biopsy material from other tissues,[4] additional evaluation to yield prognostic, personalised information is not undertaken.

Chronic liver disease (CLD) represents a significant global health burden. In progressive liver injury, fibrotic neomatrix production, coordinated by hepatic myofibroblasts, exceeds native matrix degradation by matrix metalloproteinases (MMPs), resulting in progressive scar accumulation. However, after cessation of injury or treatment of the primary disease, the liver has capacity for profound recovery. Data from well characterised animal models[5,6] and studies in human CLD,[7,8] have demonstrated the potential reversibility of hepatic fibrosis. Moreover, histological regression of fibrosis is associated with a reduction in portal hypertension and improved clinical outcomes.[9–11] The factors limiting reversibility of fibrosis are less well understood, particularly in human CLD. Such factors could be important in predicting future decompensation events (e.g. variceal bleeding, ascites) or persistent hepatocellular carcinoma (HCC) risk,[12,13] and in the selection of patients for potential antifibrotic trials.

Elastin is an extracellular matrix (ECM) protein conferring elastic recoil to tissues. It is extremely stable *in vivo* due to cross-linking and extreme hydrophobicity. Elastin is only a minor ECM component in normal liver, but it is actively synthesised as its soluble precursor, tropoelastin, by hepatic myofibroblasts in human fibrotic liver.[14] Covalent cross-linking of tropoelastin monomers results in an insoluble, mature elastin polymer that renders accumulated scar ECM more resistant to degradation, limiting the reversibility of fibrosis.

Evidence for elastin turnover *in vivo* comes from studies showing elevated serum levels of MMP-mediated elastin breakdown fragments[15] and urinary concentrations of markers of degradation of mature cross-linked elastin (desmosine and isodesmosine) in patients with cirrhosis.[16]

Furthermore, these urinary biomarkers correlated with liver fibrosis scores in biopsies from patients with CLD secondary to hepatitis C virus (HCV) and alcohol. Elastin turnover has also been studied longitudinally in liver biopsies from 21 patients with chronic viral hepatitis,[17] indicating that deposition of elastic fibres occurred concomitantly with the formation of thick collagen bands. This is consistent with other studies showing that older scars in liver biopsy specimens can be identified by their elastin content.[18–20] More recently, hepatic elastin content has been shown to be associated with subsequent progression to development of HCC in a cohort of patients with advanced fibrosis related to HCV.[21]

We hypothesised that the elastin content of fibrotic ECM in advanced CLD varies between individuals, and that hepatic elastin content may predict the occurrence of adverse clinical events. We have developed a robust, reproducible method that utilises existing biopsy material to quantify hepatic elastin and predict poor clinical outcome. Extracting additional information from biopsy material also favourably shifts the risk-to-benefit ratio of the procedure.

## Materials and Methods

### Patient cohorts

#### *Trent HCV biopsies*

The study cohort was derived from a single centre (Nottingham) within the Trent Study of Patients with Hepatitis C Virus Infection, a prospective observational study designed following the natural history of HCV[22]. Patients underwent liver biopsy as part of routine clinical care and consented for tissue surplus to diagnostic purposes to be used for research. ALBI scores were calculated to assess the severity of liver dysfunction[23]. The Trent Study of HCV was approved by the regional ethics committee (MREC 98/3/55).

To enrich the study for a future end-point of clinical outcomes, we selected patients who had both advanced liver fibrosis and evidence of progressive disease. The inclusion criteria for elastin evaluation were:

- Active chronic HCV without sustained virologic response to therapy before or after biopsy.
- Biopsy performed before 2011, allowing time for clinical outcomes to develop.
- Biopsy assessed as Ishak Stage  $\geq 5$  by an independent histopathologist blinded to other clinical information.
- Adequate tissue remaining in the block available for elastin immunohistochemistry.
- No clinical outcome before biopsy.

#### *Lothian explant study samples*

For technical validation of elastin staining using samples from a different centre stained in an independent laboratory, non-hilar sections from human explant liver were obtained by application to the Lothian NRS Human Annotated Bioresource (ethical review number 15/ES/0094). Samples were of mixed aetiology: 7 cirrhotic (2 alcohol-related liver disease, 1 primary sclerosing cholangitis, 2 primary biliary cholangitis, 1 HCV, 1 cryptogenic) and 1 non-fibrotic (acute liver failure without fibrosis, attributed to drug-induced liver injury).

#### *Histopathology*

##### *Quantitative histology and digital image analysis using the Trent HCV study biopsies*

Four micron sections from biopsies were stained with picrosirius red (PSR), as described,[5] for Ishak[24] and Laennec[25] scoring and quantification of liver fibrosis.

To identify elastin, four micron sections were stained using a commercially-available rabbit IgG polyclonal antibody to elastin (ab21610, Abcam, Cambridge, UK). Primary antibody was used at a

dilution of 1:200 (15 minutes) on Leica Bond Max stainers after antigen unmasking with Epitope retrieval solution 2 (EDTA-based pH 9.0, AR9640, Leica Microsystems, Milton Keynes, UK). Staining was visualised with the Bond Polymer Refine Detection kit (DS9800, Leica Microsystems, Milton Keynes, UK).

For biopsies from the Trent Study cohort, whole slide images of PSR-stained sections and sections stained for elastin were acquired at a x20 magnification (NanoZoomer, Hamamatsu Photonics, Shizuoka, Japan) and split manually into smaller tiles. Post-acquisition analysis was performed using an ImageJ[26] plugin created in-house, employing statistical colour modelling to threshold images,[27]. No manual curation or masking of structures was undertaken before analysis.

Quantification of elastin immunopositivity was repeated independently at a second centre using the same raw whole-slide images. Images were split using ndpisplit[28] into tiles of x5 magnification before the application of a classifier that had been generated by a specialist liver histopathologist using the machine learning WEKA plugin in FIJI;[29,30]. All analysis was undertaken blind to all clinical and histological data.

#### *Lothian liver explant samples*

Additional work was undertaken at a second UK liver centre using alternative non-automated staining protocols. Four micron sections of explanted cirrhotic liver were stained both with (Verhoeff's) Elastic van Gieson (EVG) and the same anti-elastin primary antibody at the same dilution (1:200). Antigen retrieval was undertaken by 15 min microwaving in EDTA pH 9.0 solution, primary antibody applied for 1 hour at room temperature, and signal amplification using a VECTASTAIN ABC HRP kit (Vector Laboratories, Peterborough, UK) per manufacturer's instructions.

## Clinical outcomes

A clinical outcome was defined as the first event recorded of: i) ascites requiring treatment; ii) variceal bleeding; iii) overt hepatic encephalopathy; iv) orthotopic liver transplantation; v) liver-related death or vi) development of HCC. Patients presenting with new onset ascites but diagnosed with HCC during investigation were recorded as HCC. Data collection ceased in 2014 and none of the patients received treatment with direct acting antiviral agents. Data from hospital records was supplemented with data from the Office of National Statistics on cause of death and cancer registrations. Patients who did not reach a clinical outcome during the follow-up period were censored at the time of either i) last seen alive without evidence of a liver-related clinical outcome, or ii) non liver-related death.

## Statistics

All data was tested for normality by Shapiro-Wilk and examination of Q-Q plots, allowing appropriate parametric or nonparametric tests to be used. Parametric data is described as mean  $\pm$  standard deviation (SD); nonparametric data is presented as median with interquartile range (IQR). Spearman's correlation coefficient ( $r_s$ ) was used to measure the strength and direction of association between two ranked nonparametric variables. Bland-Altman (BA) plots and intraclass correlation coefficients were used for additional comparison of elastin quantification methods. Difference in time to outcomes was assessed using the log-rank (Chi-square) test. Cox regression analysis was used to determine factors associated with the time to clinical outcomes. Statistical analysis was performed using IBM SPSS Statistics 22 and the RStudio implementation of R[31].  $p < 0.05$  was considered statistically significant.



## Results

### Development, technical validation and reproducibility of elastin staining

Elastin and PSR content in the Trent HCV samples were quantified using the previously described ImageJ plugin[27] (figure 1 a,b); patient characteristics of the cohort are shown in Table 1. Median elastin content was higher in biopsies classified as Ishak stage 6 compared to stage 5 (figure 1 c), although the difference in median content (independent samples median test  $p = 0.27$ ) or distribution (Mann Whitney U  $p = 0.064$ ) was not significant. There was a wide range of elastin content in biopsies from both Ishak categories; Ishak stage 5 median content 2.51% (IQR 2.07-4.36; minimum 1.44, maximum 8.05); Ishak stage 6 median content 3.61% (IQR 2.65-4.52; minimum 1.69, maximum 11.19).

The difference in PSR content between Ishak stage 5 and 6 was significant (figure 1 d;  $18.80 \pm 11.52\%$  versus  $30.57 \pm 11.24\%$ ; Welch two sample t-test,  $t = -3.46$ ,  $df = 42.46$ ,  $p = 0.0012$ ). There was a statistically significant, moderate positive correlation between elastin content and collagen content (figure 1e;  $r_s = 0.58$ ,  $p = 0.000047$ ).

For technical validation of elastin staining and quantification, elastin was quantified from the same whole slide images by an independent observer blind to all data using an alternative method of image classification (WEKA). There was very strong rank correlation of elastin quantified by both methods (figure 2 a,b;  $r_s = 0.87$ ,  $p < 0.00001$ ). However, as expected from the raw values, absolute agreement between methods showed systematic proportional differences on the Bland-Altman plot (supplementary figure 1), and poor agreement by intraclass correlation (one-way;  $ICC(1) = 0.098$ ,  $p = 0.258$ ).

To confirm reproducibility of immunohistochemical detection of elastin in fibrotic liver and supportive tinctorial staining, sections from native explant hepatectomies, from a spectrum of

primary aetiology, and from a single non-fibrotic partial hepatectomy, were obtained at a second UK liver centre and stained in a different laboratory using a separate batch of the same primary antibody. A manual protocol that did not require an automated stainer, with alternative signal amplification, was used. In parallel, tinctorial identification of elastin by EVG staining was undertaken on sections from the same block (figure 2 c) to confirm elastin identification.

Elastin was evident and quantified in fibrous scars of cirrhotic explants (median cirrhotic elastin content 4.7%, IQR 2.2-9.3; non-fibrotic elastin content 0.2%, supplementary figure 2), with clear histological spatial equivalence between elastin immunopositivity and dark blue/black elastin tinctorial staining on EVG. Staining intensity was reduced compared with automated staining. This demonstrated broad aetiology-agnostic relevance and inter-laboratory applicability of antibody-based image analysis to quantify elastin.

#### Elastin content can predict adverse clinical outcome in patients with advanced fibrosis.

We hypothesised that patients with higher elastin content, independent of Ishak stage, were at greater risk of development of adverse clinical outcomes. Seventeen patients (57%) progressed to a liver-related clinical outcome in the follow-up period after liver biopsy, median follow-up 5.8 years (IQR 3.0-8.3). Recorded outcomes were: ascites (n=8), variceal bleed (n=1), liver-related death (n=2), HCC (n=5); one patient presented with ascites and encephalopathy simultaneously. None of the patients were transplanted.

Factors associated with the time to subsequent clinical outcomes were determined (Table 2). Using Cox regression analysis, only elastin content and alkaline phosphatase (ALP) were significant in univariate analysis; Ishak stage and PSR (collagen) content were not predictors of adverse outcome within this cohort. ALP remained significant in multivariate analysis (ALP hazard ratio 1.009, 95% CI 1.0033-1.015,  $p = 0.0021$ ; elastin hazard ratio 1.123, 95% CI 0.891-1.402,  $p = 0.306$ ).

The median elastin content by primary quantification method for the cohort was 3.4% (IQR 2.2-4.7). 11/15 patients (73%) with greater than median elastin content progressed to a clinical outcome, compared to only 6/15 (40%) of those with elastin content below the group median. The difference in time to outcomes was significant (Log rank: Chi square 3.98;  $p = 0.046$ , figure 3 a). Elastin content quantified by WEKA classification (median 7.5, IQR 5.9-14.4) was also predictive of clinical outcome; a calculated optimum cut-off value of 6.2% also gave a significant difference in time to outcome (Log rank: Chi square 4.6;  $p = 0.0312$ , figure 3 b).

We performed a subgroup analysis to ascertain whether elastin was a predictor of HCC as a single outcome in our cohort. Only five patients (17%) developed HCC during the follow-up period. Elastin content was not statistically associated with development of HCC (Cox regression: Exp(B) 1.00 [0.61-1.62];  $p = 0.99$ ).

## Discussion

In this study, we have developed a method to obtain clinically-useful prognostic information from existing liver biopsy material, using an anti-elastin primary antibody and digital image analysis to detect and quantify elastin content in advanced CLD, and confirmed using independent classification methods. Histological equivalence in identification of elastin by both immunohistochemistry and a well characterized tinctorial stain (EVG) was demonstrated. The use of an antibody to identify elastin, based on initial animal studies by the Edinburgh group,[18,32] has the advantage of conferring specificity that may be needed in antifibrotic studies but disadvantage of adding additional complexity and cost compared with tinctorial stains.

As proof-of-concept, we chose a group of patients with HCV and advanced CLD lacking sustained virologic response to standard contemporaneous treatment (i.e. Pegylated interferon and ribavirin). This restricted cohort was chosen to allow study of patients with rapid fibrosis progression and the highest rate of adverse clinical outcomes. A recent study demonstrated that elastin is associated

with HCC development [21] in patients with advanced fibrosis. However, this was over a limited follow up period, and elastin as a predictor of decompensated disease was not evaluated. We demonstrated significant variation in elastin content in patients with advanced CLD, and shown that elastin content can be used as a tissue biomarker of adverse liver-related outcomes. In contrast, neither Ishak stage nor PSR (collagen) content predicted outcome in this cohort.

Liver biopsy remains an important clinical diagnostic tool[3] but there is a clear obligation to extract as much information from biopsy material as possible, given associated risks.[1] The data provides encouragement to examine elastin in large, prospective cohorts of advanced liver fibrosis with continuing liver injury. Demonstration of elastin in different aetiologies of human disease suggests potential utility across all CLD but this requires evaluation in disease-specific cohorts.

Staging of fibrosis using available ordinal scoring systems is subject to considerable observer variability and generates crude ordinal data, indicating a need for alternative methods of evaluation. Quantification of fibrosis from biopsy material using PSR-stained sections addresses some of these problems but is not routinely undertaken in clinical practice. In our study, establishment of a laboratory-specific standard allowed elastin content to be used as a tissue biomarker predicting individual outcome from the clinically-indicated biopsy with minimal operator input once standards were established. With minimal cost and from a standard, clinically-indicated liver biopsy, quantification of elastin could be incorporated as part of more nuanced histological assessment, for example stratifying patients unlikely to benefit from putative antifibrotic therapy, or identifying those requiring earlier referral for assessment for liver transplantation. The ability to obtain this information from whole-slide images, which can be centrally verified and easily accessed, is of additional value to clinical trials wishing to ensure appropriate patients are included for novel therapies.

The resistance of hepatic elastin to degradation suggests that elastin content may influence the balance of fibrogenesis and reversibility in favour of scar accumulation. Progressive scar formation with consequent bridging of vascular structures leads to portal hypertension. The non-HCC adverse liver-related outcomes in this proof-of-concept study are related to portal hypertension. Increased hepatic elastin may be a consequence of aberrant hepatic blood flow and sinusoidal pressure; in the context of liver injury, it has been suggested that elastin is deposited by portal fibroblasts to limit damage caused by increased biliary ductal pressure[33] and that elastin deposition is a result of tensile and shearing effects.[34] It is also recognised that strained ECM leads to greater production of fibrotic glycoproteins than a relaxed matrix.[35–37] Given the focus on predicting clinical outcomes in advanced CLD, the role of elastin in homeostasis or during earlier stages of fibrogenesis has not been examined.

This was an initial proof-of-concept study and therefore has inherent limitations. A single-aetiology cohort has been studied; there is evidence that matrix composition or amount may vary between diseases[38] so further work with additional cohorts of alternative aetiologies and similarly prolonged follow up is required. The Cox regression analysis should be interpreted cautiously given the size of the pilot cohort studied meant that recognised prognostic factors were not identified as significant by univariate analysis. Additionally, it is clear that inter-laboratory differences in staining protocols and performance, demonstrated by the differences between manual and automated staining in our study, mean that prescriptive application of the cut-off value derived from this study is inappropriate, and further work to allow laboratory protocol harmonisation would be required before routine application in practice is merited. Quantifying elastin from EVG stained sections that are more readily and reproducibly available is an obvious means of minimising inter-laboratory staining variation to develop a more broadly applicable tool.

Elastin accumulates in fibrotic livers regardless of the underlying aetiology, is a key determinant of irreversibility, and its presence may predict the development of clinical outcomes independently of

collagen fibres. Hepatic elastin quantification should be evaluated further in larger studies to establish its potential role in clinical decision-making and selection of patients for therapeutic trials.

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## Legends

Figure 1 - Quantification of elastin and fibrosis in biopsies of advanced (Ishak stage  $\geq 5$ ) hepatitis C virus infection. Biopsies from patients with chronic hepatitis C virus infections and advanced stage (Ishak  $\geq 5$ ) were stained with an antibody for elastin or with picrosirius red (PSR), representative whole-slide images of a single case (A and B, respectively; main scale bars 5 mm, inset 100 microns). Elastin and PSR content were quantified by image analysis; there was no significant difference in elastin content between Ishak stage 5 and 6 (C; 2.51% (IQR 2.07-4.36) versus 3.61% (IQR 2.65-4.52), respectively, independent samples median test  $p = 0.27$ ) but there was a significant difference in PSR content between stages (D;  $18.80 \pm 11.52\%$  versus  $30.57 \pm 11.24\%$ , respectively, Welch two sample t-test  $p = 0.0012$ ). There was a moderate positive correlation between elastin content and collagen

content (E;  $r_s = 0.58$ ,  $p = 0.000047$ , regression line from fitted linear model and 95% confidence intervals).

Figure 2 - Independent validation of immunohistochemical elastin quantification and technical validation. Elastin content in original whole-slide images were independently quantified using an alternative machine learning based approach (A) to classify images into elastin immunopositive (lilac) and immunonegative (green) tissue or blank (red) pixels. There was very strong rank correlation of elastin quantified by both methods (B;  $r_s = 0.87$ ,  $p < 0.00001$ ). Explant liver from a second liver centre was independently stained with a different batch of the same primary anti-elastin antibody using a non-automated protocol from cases with a spectrum of primary aetiologies to demonstrate centre-and disease-agnostic applicability (C; representative images); sections from the same blocks were also tinctorially stained to identify elastin (elastic van Gieson, EVG) and demonstrate spatial equivalence in areas of fibrosis and internal elastic lamina of arteries as an internal positive control). Scale bars 100 microns.

Figure 3 – Elastin content functions as a tissue biomarker predictive of adverse liver-related events. (A) Liver biopsies with elastin content greater than the median (3.4%) by primary classification more rapidly developed an adverse liver-related event compared with those with elastin content below this value; the difference in time to outcomes was significant (Log rank: Chi square 3.98;  $p = 0.046$ ). (B) Using WEKA quantification, biopsies with elastin content greater than the optimum calculated cut-off (6.2%) more rapidly developed an adverse liver-related event compared with those with elastin content below this value; the difference in time to outcomes was significant (Log rank: Chi square 4.60;  $p = 0.0312$ ).

Table 1. Patient characteristics of the Trent HCV study cohort. Patient clinical, biochemical and histological characteristics (IQR, interquartile range; BMI, body mass index; HCV, hepatitis C virus;

ALP, alkaline phosphatase; GGT; gamma-glutamyl transferase; ALT, alanine transaminase; PSR, picrosirius red; ALBI, albumin-bilirubin score).

Table 2. Predictors of liver-related clinical outcomes. Elastin content and serum ALP are the only significant predictor of a liver-related clinical event, determined by univariate Cox regression analysis (\* Genotype 1 used as reference, analysis performed only comparing genotypes 1 and 3; ^ Ishak stage 5 used as reference standard; \*\*grade 0 steatosis used as reference standard; CI, confidence interval; BMI, body mass index; HCV, hepatitis C virus; ALP, alkaline phosphatase; GGT; gamma-glutamyl transferase; ALT, alanine transaminase; PSR, picrosirius red; ALBI, albumin-bilirubin score).

Supplementary figure 1. Bland-Altman plot comparing elastin quantification by primary and WEKA methods. Systematic proportional disagreement between methods is evident. Horizontal lines indicate mean, and upper and lower limits of agreement (with 95% confidence intervals of each).

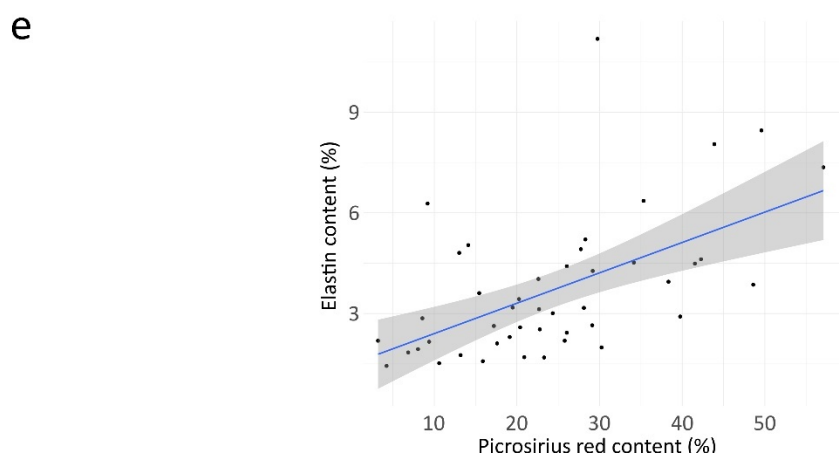
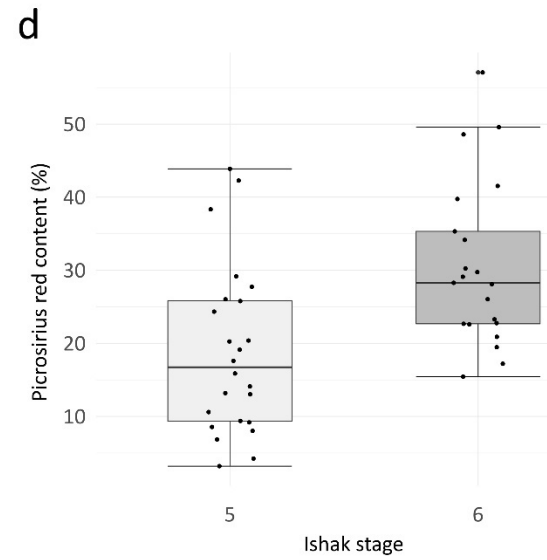
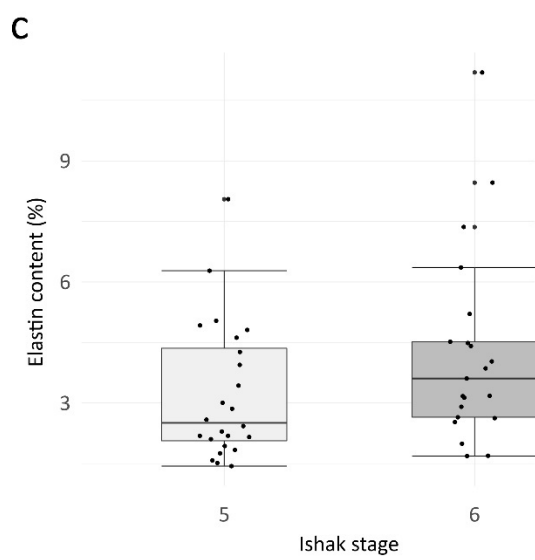
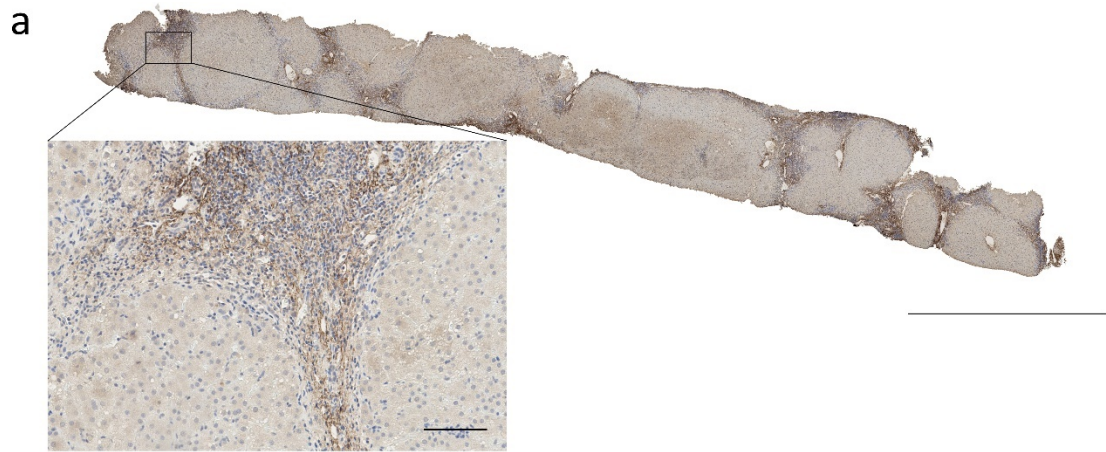
Supplementary figure 2. Elastin content of cirrhotic explant and non-fibrotic resection cases, by WEKA quantification after laboratory-specific classifier training. Data are represented as individual points with median (centre line), first and third quartiles (lower and upper box limits), 1.5x interquartile range (whiskers).

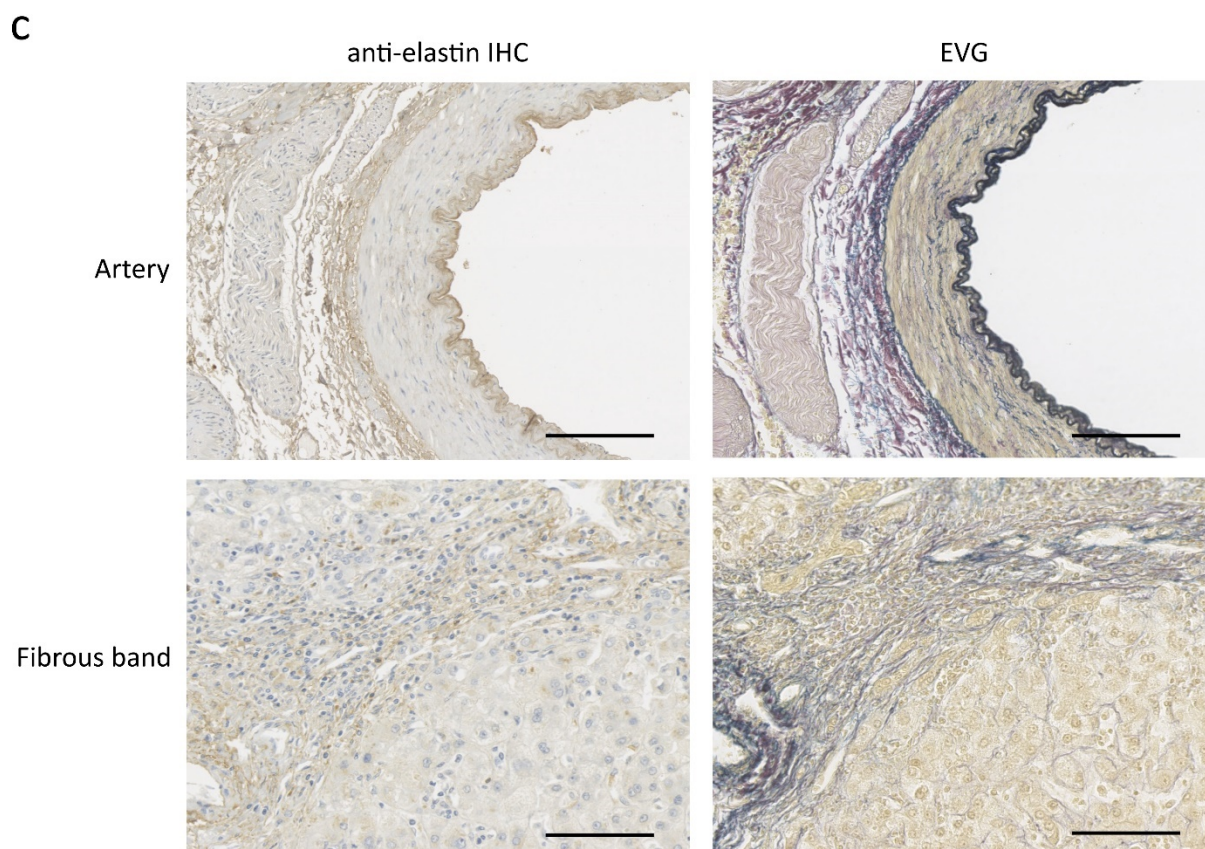
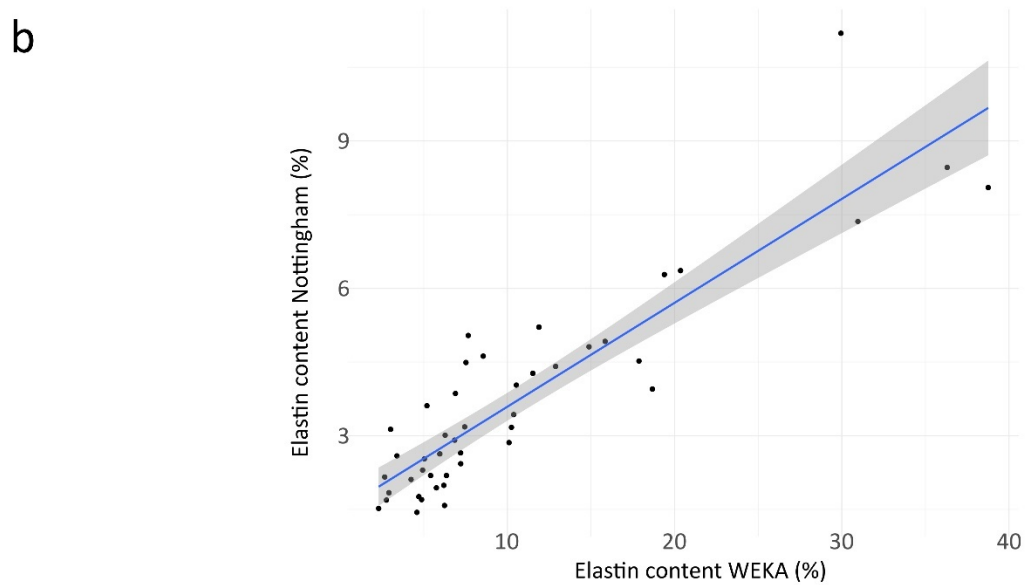
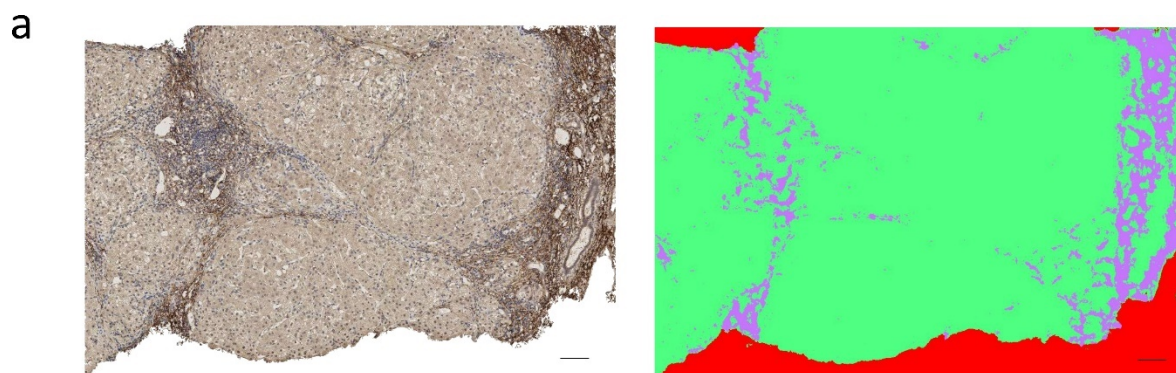
Variable	Median	IQR	Number
Age (years)	49	42 - 54	30
Gender - male			23 (77%)
BMI (kg/m <sup>2</sup> )	27	25 - 30	27
Estimated duration from infection to biopsy (years)	28	20 - 31	27
Past heavy alcohol use			17 (57%)
Heavy alcohol use (>50/week)			9 (30%)
HCV Genotype			
1			10 (33%)
2			0
3			17 (57%)
4			1 (3%)
Albumin g/L	36	38 - 40	27
Bilirubin $\mu$ mol/mL	12	9 - 16	29
ALP u/L	113	86 - 220	29
GGT u/L	166	82 - 339	29
ALT u/L	127	85 - 201	29
Ishak stage 5			16 (53%)
Ishak Grade			
0-6			13 (43%)
7-12			16 (53%)
13-18			1 (3%)
Laennec stage			
3			3 (10%)
4A			3 (10%)
4B			10 (33%)
4C			14 (47%)
PSR (collagen) (%)	22.8	15.6 - 29.9	30
Elastin (%)	3.4	2.2 - 4.7	30
Biopsy length (mm)	14.5	11.8 - 18.0	
Number of portal tracts	15	13 - 20	
ALBI score	-2.6	-2.77 - -2.39	27

Table 1

Variable	Exp (B)	95% CI	P-value
Age (years)	1.059	0.989-1.134	0.100
Gender (male)	0.405	0.137-1.198	0.103
BMI (kg/m <sup>2</sup> )	1.093	0.961-1.245	0.177
Estimated duration from infection to biopsy (years)	1.011	0.955-1.071	0.704
Past heavy alcohol use	1.371	0.464-4.049	0.568
Heavy alcohol use (>50 units/week)	1.980	0.707-5.544	0.194
Genotype*	1.889	0.588-6.070	0.286
Albumin g/L	0.947	0.868-1.034	0.947
Bilirubin $\mu$ mol/ml	1.031	0.960-1.107	0.404
ALP u/L	1.010	1.004-1.015	0.001
GGT u/L	1.001	0.999-1.003	0.279
ALT u/L	0.994	0.987-1.001	0.088
Ishak stage^	1.307	0.500-3.415	0.584
Laennec stage			
3 (Reference)			
4A	0.490	0.030-7.961	0.616
4B	0.661	0.068-6.399	0.721
4C	2.058	0.265-16.005	0.490
Ishak grade	0.890	0.334-2.372	0.816
0-6 vs 7-18			
PSR (collagen) (%)	1.010	0.947-1.078	0.758
Elastin (%)	1.226	1.007-1.493	0.042
ALBI score	1.836	0.759-4.439	0.178

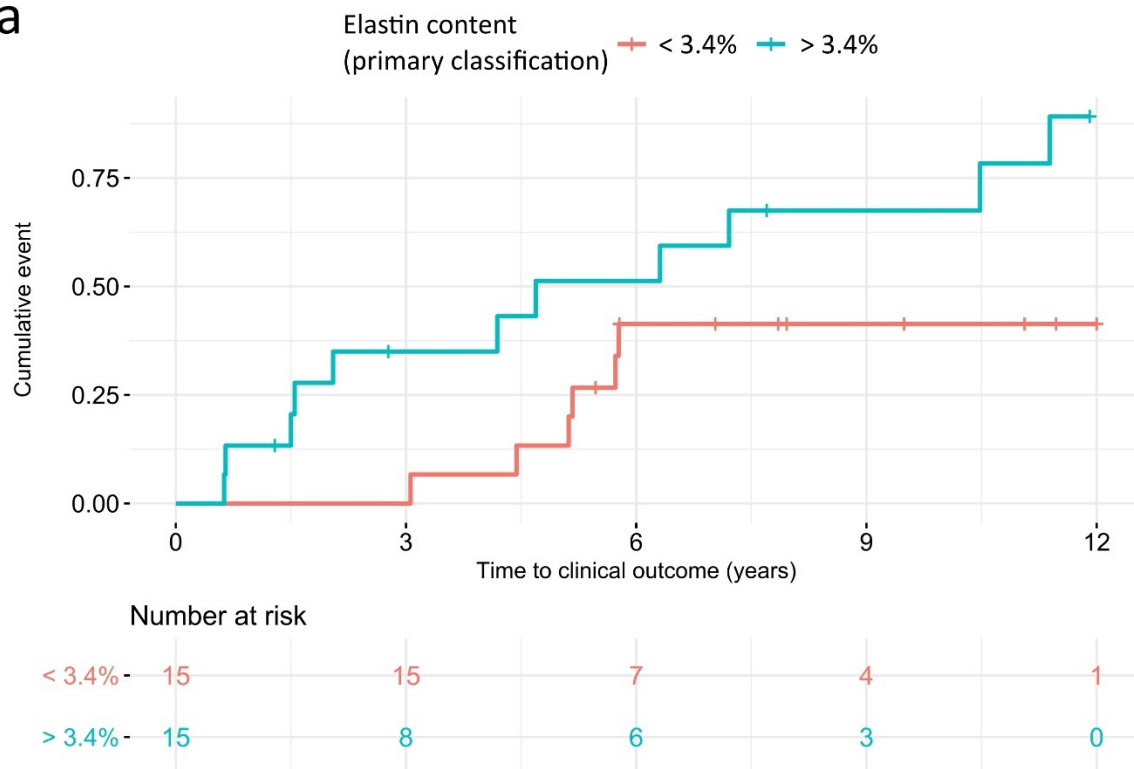
Table 2







a



b

